nature | methods

Engineering a polarity-sensitive biosensor for time-lapse imaging of apoptotic processes and degeneration

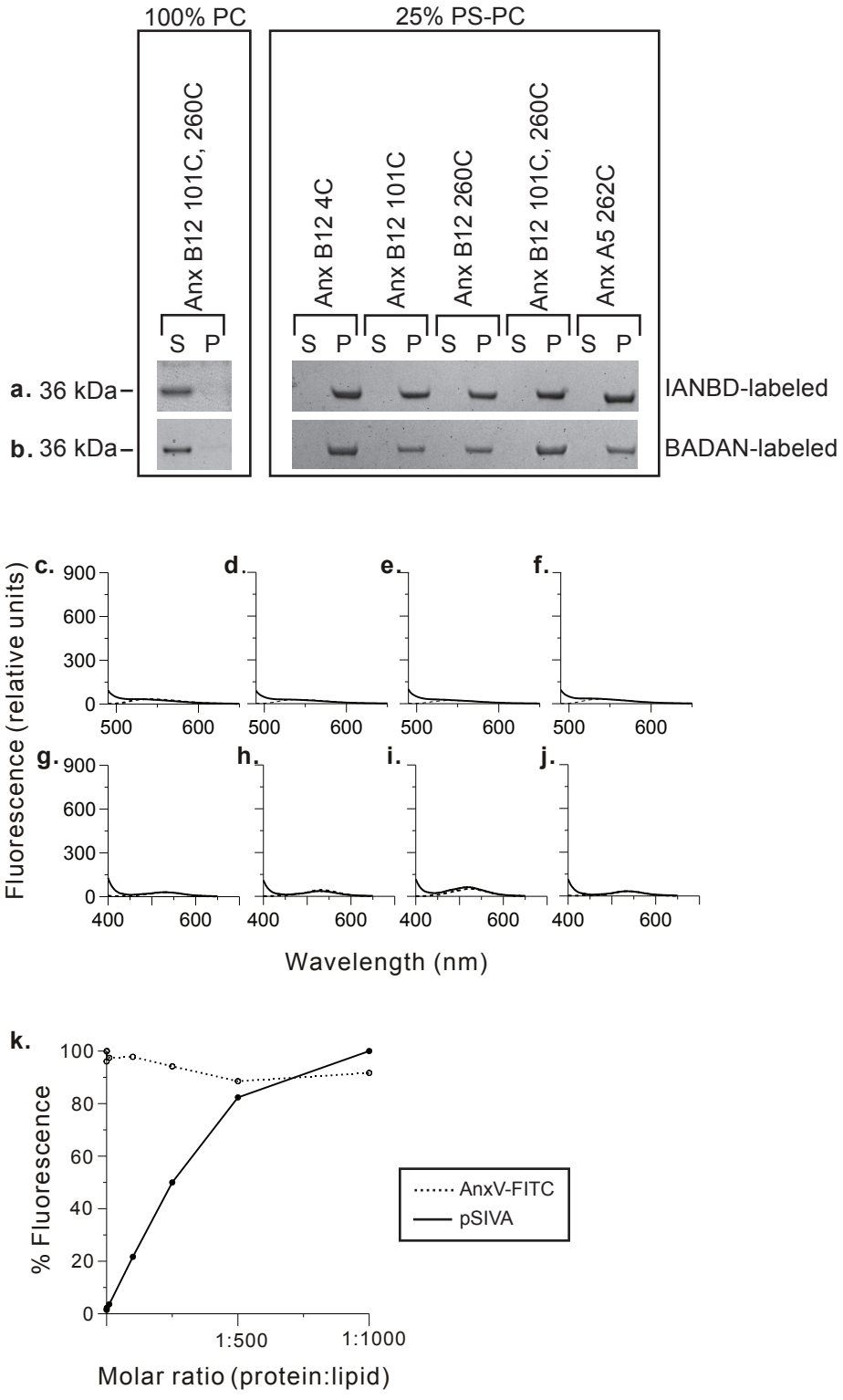
Yujin E Kim, Jeannie Chen, Jonah R Chan & Ralf Langen

Supplementary figures and text:

Supplementary Figure 1	Additional controls for pSIVA fluorescence and membrane-binding.
Supplementary Figure 2	Additional examples of apoptotic COS-7 cells detected by $pSIVA_m$.
Supplementary Figure 3	The staining pattern of $pSIVA_m$ and neurofilament on NGF-deprived (20 h) DRG neurons.
Supplementary Table 1	Sequences of oligos used to make cysteine mutants.

Note: Supplementary Videos 1–4 are available on the Nature Methods website.

Supplementary Fig. 1

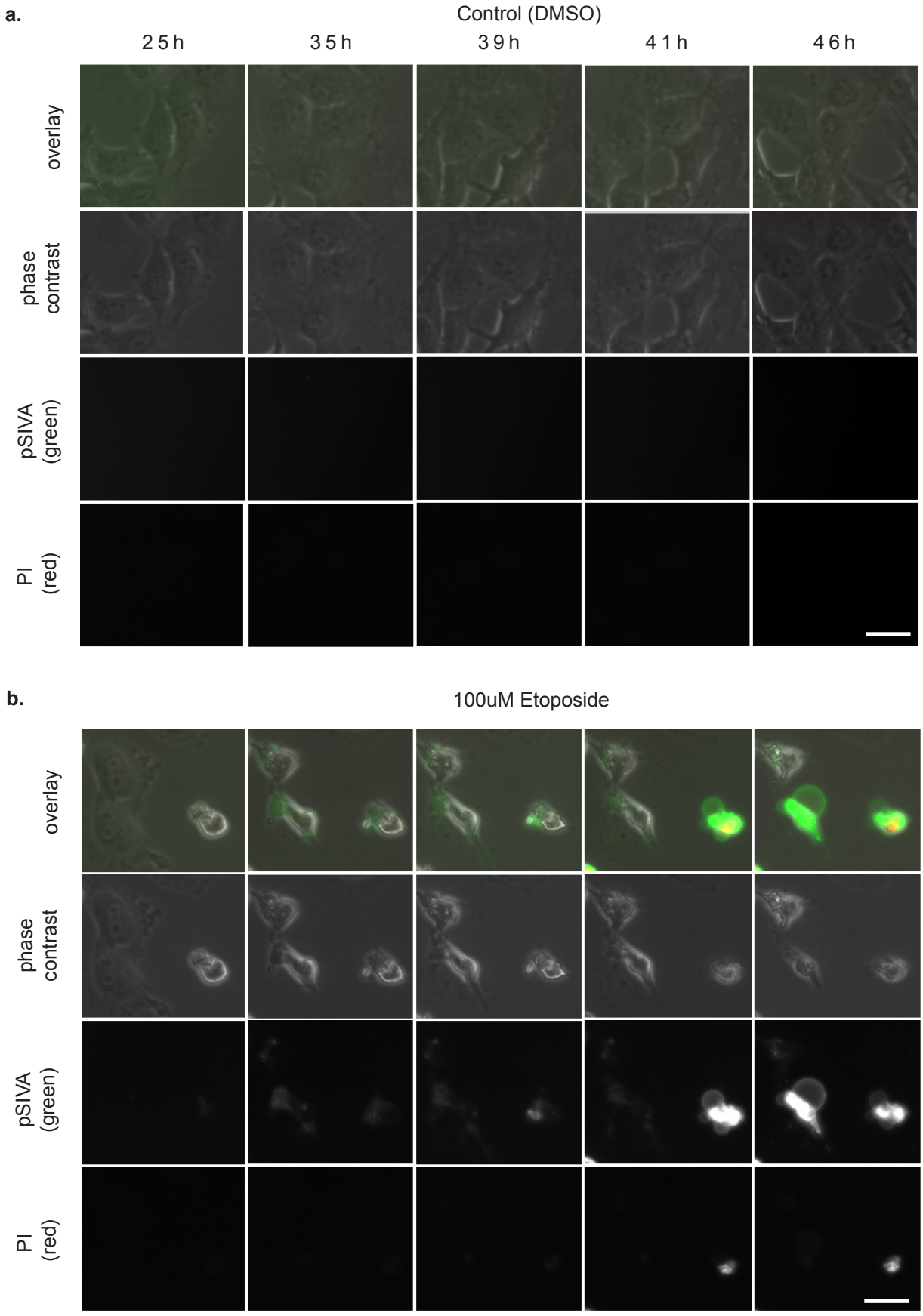


Supplementary Fig. 1

Additional controls for pSIVA fluorescence and membrane-binding.

A co-sedimentation assay of the various IANBD (**a**) and BADAN (**b**) labeled annexin proteins with liposomes indicates that labeling at the various sites does not affect membrane-binding affinity or specificity. All of the proteins were associated with the lipid pellet (P) when incubated with PS-containing vesicles. When incubated with vesicles composed of 100% PC all of the proteins remained in the supernatant (S). **c-j** shows the fluorescence intensities of the different pSIVA variants in solution (dotted lines) and the fluorescence intensities in the presence of 100% PC vesicles (solid lines). The excitation wavelength was set to 478 nm and fluorescence emission intensities were measured for IANBD labeled annexins: **c**) AnxB12 101C-IANBD, **d**) AnxB12 260C-IANBD, **e**) AnxA5 262C-IANBD and **f**) AnxB12 101C-, 260C-IANBD. The excitation wavelength was set to 380 nm and the fluorescence emission intensities were measured for BADAN labeled annexins: **g**) AnxB12 101C- BADAN, **h**) AnxB12 260C-BADAN, **i**) AnxA5 262C- BADAN and **j**) AnxB12 101C-, 260C- BADAN. The only measurable changes observed between the comparison of fluorescence intensities in the absence or presence of PC vesicles was due to lipid scatter. **k,** To provide a comparison of pSIVA (Anx B12 101C-, 260C-IANBD) to AnxV-FITC, 1 μM annexin protein was incubated with varying amounts of PS-containing vesicles (1:1000, 1:500, 1:250, 1:100, 1:50. 1:10, and 1:1 molar ratios of protein:lipid). The maximum fluorescence emission intensities measured at the different protein:lipid ratios for anxB12-101C-,260C-IANBD (excitation max. = 478 nm, emission max. = 525) was compared with AnxV-FITC (excitation max. = 485 nm, emission max. = 520 nm). As expected for pSIVA, the fluorescence intensity increased with increasing amounts of PS-containing vesicles, indicating that increasing fluorescence intensities correlates with increasing membrane-binding. No significant change in the overall fluorescence intensity was observed for AnxV-FITC when increasing the amount of PS-containing vesicles.

Supplementary Fig. 2



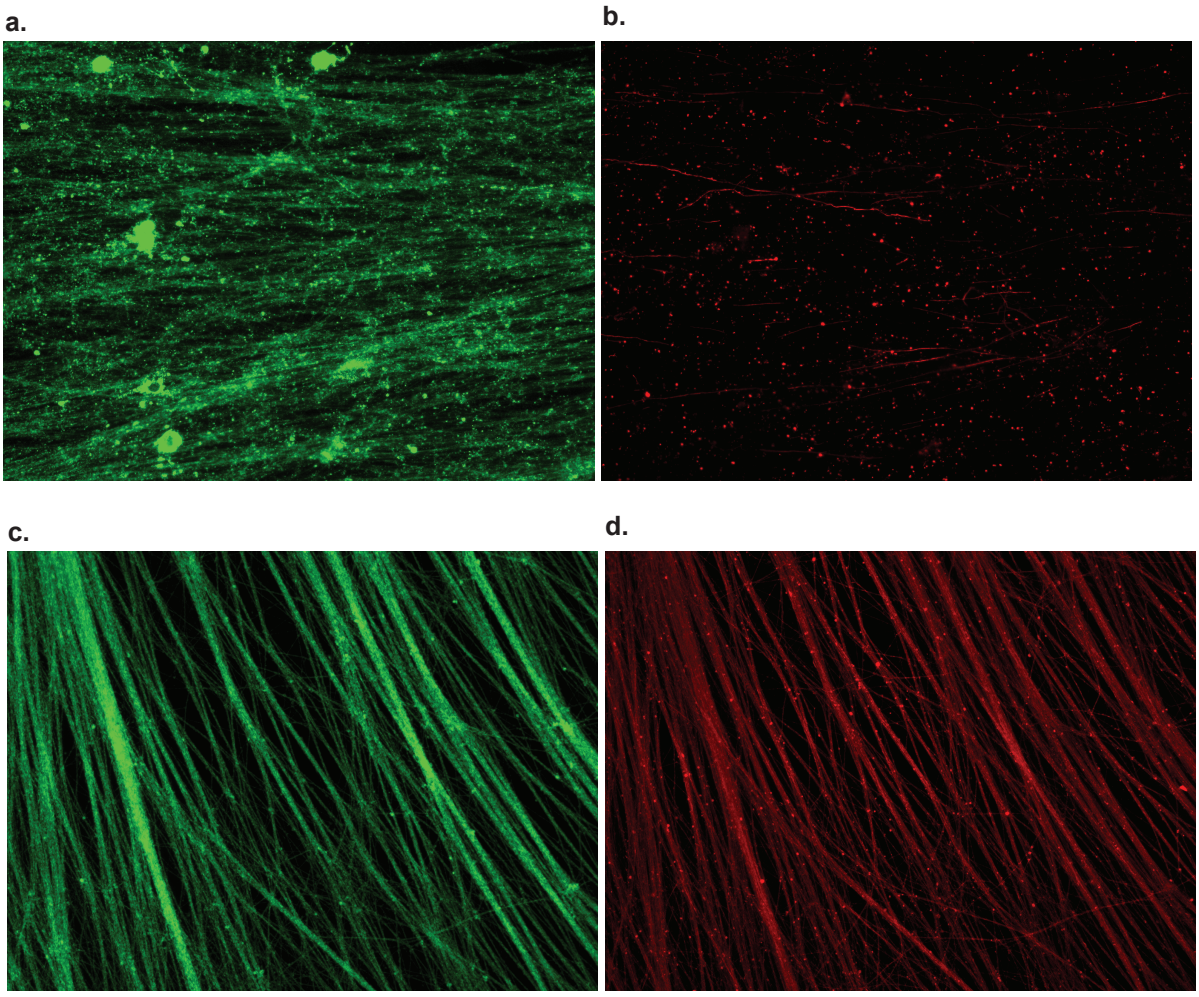
Nature Methods: doi:10.1038/nmeth.1405

Supplementary Fig. 2

Additional examples of apoptotic COS-7 cells detected by pSIVA.

a, COS-7 cells monitored under physiological conditions and **b**, in the presence of the apoptotic factor, etoposide. Green fluorescence indicates pSIVA binding to the PS exposed on the outer leaflet of the plasma membrane, and red fluorescence indicates propidium iodide (PI) staining of nuclei during later stages of apoptosis, with loss of plasma membrane integrity. (Scale bars, 40 μm)

Supplementary Fig. 3



Supplementary Fig 3.

The staining pattern of pSIVA and neurofilament on NGF-deprived (20 h) DRG neurons. Live-staining (unpermeabilized) of DRG neurons was compared with staining of permeabilized neurons. Similar to what was observed in the live-cell imaging experiments, pSIVA staining of axons which were not permeabilized resulted in a punctate staining pattern (**a**)**.** Neurofilament staining was excluded from the majority of axons (**b**), indicating that the pSIVA signal (**a**) arises from binding to exposed PS on axons with membrane integrity still intact. In contrast the staining pattern of permeabilized cells with pSIVA was uniform throughout the axon (**c**), in agreement with the more abundant and uniform distribution of PS on the inner leaflet of the plasma membrane. For comparison, neurofilament staining of permeabilized axons is shown in **d**.

Supplementary Table 1

mutation	oligo sequence
AnxB12: Q4C	5'-GAA TAA ACC ATG GTT GTT TGT GGA ACA GTT AAA CCA CAT G-3'
AnxB12: L101C	5'-GCT ATG AAG GGG TGT GGA ACT GAT GAA AAC-3'
AnxA5: A262C	5'-GCT ATG AAG GGA TGT GGG ACA GAT GAT CAT ACC C-3'
AnxA5: C316A	5'-GCT CTT CTG CTG CTC GCT GGA GAA GAT GAC-3'